5 A new cryptic *CFTR* exon in mild CF

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So far, over 1500 *CFTR* gene mutations have been described responsible for CF and CFTR-related disorders. Screening for large gene rearrangements has improved identification of CF alleles but a number of cases remain unsolved, making genetic counselling difficult, particularly when the diagnosis is uncertain. It has been hypothetised that unidentified CF mutations may lie in introns which are not routinely investigated. Such events have been rarely reported, such as 3849+10kbC>T and 1811+1.6kbA>G.

We report the case of a French 25 y woman diagnosed at 4 y with disseminated bronchiectasis, pancreatic sufficiency and borderline sweat tests. Screening of the 27 exons including search for gene rearrangements only identified F508del in heterozygosity. Investigation at the mRNA level was then performed from nasal epithelial cells.

An abnormal, longer *CFTR* mRNA was detected, corresponding to a 97bp insert between exons 6b and 7. This fragment matches a 101bp sequence of intron 6b deleted of 4bp, which starts 1061bp upstream exon 7 and is flanked by the consensus donor and acceptor splice sites. A new stop codon is created within the inserted sequence. The amount of abnormal *CFTR* mRNA of the patient was estimated to be 20% of the wild type, which suggests the abnormal splicing is due to partial activation of a cryptic splice site whose impact could be considered as mild. This hypothesis is supported by the fact that the patient does not suffer from severe CF. Studies at the genomic level are still ongoing to identify the mutation which leads to the partial activation of a cryptic splice site and explains the creation of a new exon.

6 Validation of an interactive multimedia programme for informed decision-making in cystic fibrosis genetic testing

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Owing to the steadily increasing number of requests for CF genetic testing, it is impossible providing classic genetic counselling sessions to all who want the test. This study evaluates the performance of a recently developed interactive multimedia software, designed to be a decision aid for CF genetic testing potential users. Twenty five participants (21M 4F) were recruited by Clinical Genetics Services in Verona and Milan. They were included in either group GC where education was provided by a Genetic Counsellor (7 individuals), or in group IC, where education was provided by the Interactive Computer programme (18 individuals). Both groups were assessed by a 10 question multiple-choice questionnaire before and after the counsellor/computer session, and the results evaluated by a two-sample t-test. Average correct answers raised from 3.71/10 before the information to 9.57/10 after the information in group GC (p 0.001), and from 4.17/10 to 9.22/10 in group IC ($p < 0.0001$); wrong answers decreased from 1.86/10 before the information to 0.43/10 after the information in group GC (n.s.), and from 2.06/10 to 0.78/10 in group IC (p 0.008); "don't know" answers changed from 4.43/10 before the information to 0/10 after the information in group GC, and from 3.78/10 to 0/10 in group IC. These preliminary results show that a multimedia programme may perform at least as well as traditional individual genetic counselling in promoting understanding about CF genetic testing.

7* The French CF Laboratory Network: seven years' experience

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The French CF Laboratory Network, composed of 35 molecular genetics laboratories and which settlement had been facilitated by the European CF Network, was recognized seven years ago by the French Ministry of Health. Ten of the laboratories received financial support for in-depth *CFTR* gene molecular analysis and a subgroup of four had additional support for management of the Network and CF clinical research.

Based on best practices guidelines for *CFTR* gene analysis, DNA samples may be processed in one or two different laboratories, depending on the indication of the test and the laboratories' level of expertise. More than 10,000 samples are studied per year (50–1500 per lab) including 200 prenatal diagnoses. The continuous share of experience on rare variants greatly helps genetic counselling.

Since 2001, six workshops were organized, convening molecular geneticists, technicians, clinicians of CF care centres and private companies. Three main topics were addressed: (1) Network functioning, discussion on best practice guidelines and report on European actions; (2) technical points including new technologies; (3) and clinical issues. A number of collaborative studies have been published and others are still ongoing, such as the spectrum of mutations in CF and CBAVD in France; the *CFTR* genotypes in patients with normal/borderline sweat tests; the incidence of CF in fetal bowel anomalies; or phenotype-genotype correlations on R117H. The active involvement of the molecular geneticists of specialized laboratories in European actions greatly contributes to the dynamism of the French Network.

8* Complete sequencing of the CFTR gene using new generation GS-FLX sequencing technology

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New generation sequencing technology has been recently introduced. However, this technology was initially developed for whole genome sequencing purposes.

We have adopted this technology for complete sequence analysis of the CFTR coding region, and its exon/intron junctions.

For a 50x coverage, only half a million nucleotides are needed for CFTR sequence analysis, i.e. 0.5% of the full capacity of the GS-FLX system. Therefore, 100–200 samples should be pooled in order to use the full capacity of the GS-FLX system. We have developed an economically feasible universal sample tagging approach allowing the pooling of 100 samples with one set of 260 primers. This compares to 6000 primers if amplicon-specific PCR primers are tagged as such.

Normally, each amplicon should be amplified individually and purified, after which the concentration of each solution is accurately determined, and an equimolar amplicon mixture is prepared for sequencing. The universal tagging approach resulted in a less dynamic range of the yield of the different amplicon products, so that the preparation of the equimolar amplicon mixture is simplified.

We are even further simplifying the PCR reactions through the development of robust multiplex PCR reactions. Indeed, 30 amplicons should be analyzed for the CFTR gene, and this can ultimately be only economically feasible if amplified in one, or a limited number, of multiplex PCR reaction(s). Specifically we have developed a robust multiplex amplification assay in which biotinylated amplicon-specific primers are locally restricted through streptavidin/biotin crosslinking.

We thus developed an assay for routine sequencing of the CFTR gene in a diagnostic setting by next generation sequencing. Moreover, this assay is readily transferable to any gene.